REMARKS

Preliminary Remarks

Reconsideration and allowance of the present application based upon the following amendment and remarks are respectfully requested. Currently 9, 12, 13, 15, 16, 18, and 21-28 are pending and remain at issue. This response is timely filed as it is accompanied by a petition for an extension of time to file in the third month and the requisite fee.

In paragraph 2 of the official action, the examiner requested translations of priority German patent applications 10043336.7 and 10126422.4 filed September 2, 2000 and May 31, 2001, respectively. The applicants submit that the certified translations of both priority documents were submitted to the Patent Office on May 16, 2004. The applicants request acknowledgement of receipt of these documents.

In paragraph 7 of the official action, the examiner objected to the applicants' amendment to the abstract because the abstract allegedly remained in an improper format. The applicants submit herewith a new abstract, which is in a single, narrative paragraph indicating the necessary source species of the invention. Accordingly, the applicants respectfully submit that the objection to the abstract has been overcome and should be withdrawn.

In paragraph 21 of the official action, the examiner objected to the amendment filed January 6, 2004 under 35 U.S.C. §132 because the incorporation of U.S. Prov. Appl. No. 60/295,009 is improper unless the provisional application is an exact duplicate of the instant application, wherein such incorporation is redundant. The applicants have amended line 2, page 1 of the specification to remove the language referred to by the examiner.

In paragraphs 23 and 24 of the official action, the examiner objected to the amendment filed January 6, 2004 under 35 U.S.C. §132 because it allegedly introduces new matter in the disclosure. Specifically, the examiner asserted that for purposes of describing the sigma factor E on page 6 of the specification, the term "RNA polymerase" is not supported in the specification as originally filed (U.S. Prov. Patent Appl. No. 60/295,009). The applicants have amended the specification at page 6, line 20 to remove the phrase "RNA polymerase." The term sigma factor E however is fully supported in the priority documents. Sigma factor E is a protein that is not an enzyme, but is part of a genus of well known proteins associated with RNA polymerase. Sigma factor E is a protein that correctly and efficiently positions an RNA polymerase to recognize particular promoters of a particular genes. Sigma factor E was well known protein isolated from several bacterial species at the

time of filing (*i.e., Escherichia coli, Streptomyces coelicolor, Mycobacterium tuberculosis, Mycobacterium bovis*; See Attachment A). In view of the foregoing amendment and remarks, this objection has been overcome and should be withdrawn.

In paragraph 25 of the official action, the examiner objected to claim 22 under 37 C.F.R. §1.75(c) as being in improper dependent form for failing to further limit the subject matter of claim 9. Specifically, the examiner asserted that the claimed subject matter of claim 22 does not further limit the claimed subject matter of claim 9. Amended claim 22 has been rewritten in independent form and no longer depends upon claim 9 thereby obviating the objection to the claim.

The applicants do not intend by these or any amendments to abandon subject matter of the claims as originally filed or later presented, and reserve the right to pursue such subject matter in continuing applications.

Patentability Remarks

Claim Rejection-35 U.S.C. §112, Second Paragraph

Claims 15 and 16

In paragraph 21 of the official action, claims 15 and 16 were rejected under 35 U.S.C. § 1.12, second paragraph, as allegedly being indefinite. Specifically, the examiner alleged that the listed genes in the claims 15 and 16 are not limited to *C. glutamicum* even though only one example of each of the listed genes are from *C. glutamicum*. The examiner also asserted that claiming the gene name along with the enzyme name is confusing. The examiner also alleged that the phrases "a protein for lysine export", "a Zwal protein", and "a Zwa2 protein" were unclear as to their metes and bounds regarding the relationship between the name and function of the protein. The examiner asserted no limitation was given to these specific proteins in the claims, and thus, it would be unclear to one of skill in the art which genus of genes would encode a protein for lysine export, for example. Finally, the examiner asserted that claim 15 does not clearly define the claimed group and suggested inserting the word "following" in front of the word "group" for clarity purposes.

Amended claim 15 is now directed to the process of claim 9, further comprising overexpressing a *C. glutamicum* gene selected from the group consisting of a gene which codes for dihydrodipicolinate synthase, a gene which codes for dihydrodipicolinate synthase, a gene which codes for glyceraldehyde 3-phosphate dehydrogenase, a gene which codes for

triose phosphate isomerase, a gene which codes for 3-phosphoglycerate kinase, a gene which codes for glucose 6-phosphate dehydrogenase, a gene which codes for pyruvate carboxylase, a gene which codes for malate-quinone oxidoreductase, a gene which codes for aspartate kinase, a gene which codes for a protein that exports lysine, a gene which codes for homoserine dehydrogenase, a gene which codes for threonine dehydratase, a gene which codes for acetohydroxy-acid synthase, and a gene which codes for dihydroxy-acid dehydratase is overexpressed.

Amended claim 16 is directed to the process according to claim 9, further comprising deleting a *C. glutamicum* gene selected from the group consisting of a gene which codes for phosphoenol pyruvate carboxykinase, a gene which codes for glucose 6-phosphate isomerase, and a gene which codes for pyruvate oxidase, is deleted. Solely to expedite prosecution and without prejudice to seeking broader claims in a continuing application, the applicants have deleted the language regarding overexpressing the genes encoding the Zwa1 and Zwa2 proteins thereby rendering this part of the rejection moot.

With regard to the *lys*E gene, the applicants have amended part (i) of claim 15 to read "a gene which codes for a protein that exports lysine." The *C. glutamicum lys*E gene is taught as being one of the genes that can be overexpressed in the specification at page 13, lines 10 and 11. At the time of filing this application, it was known that the *C. glutamicum lys*E gene encoded a transporter protein that specifically exported L-lysine from the cell. A *lys*E deletion mutant accumulates a high concentration of L-lysine. By overexpressing the *lys*E gene, L-lysine is exported at a rate that is five times the rate observed in a wild type *C. glutamicum* cell (See Attachment B, Vrljic *et al.*, *Molecular Microbiology* 22:815-826 (1996)).

Accordingly, the applicants submit that the phrasing of part (i) of claim 15 clearly defines the particular gene, which encodes a protein for lysine export. In lieu of the teachings of the specification on page 13, lines 10 and 11, and original claim 15, part (i) of claim 15 clearly indicates the function and structure of the *lys*E gene of *C. glutamicum*. In view of the foregoing amendment and remarks, the applicants submit the rejection of claims 15 and 16 under 35 U.S.C. §112, second paragraph, for indefiniteness as described above, has been overcome and should be withdrawn.

Claims 9, 12, 13, 15, 16, 18, 21, and 22

In paragraphs 26 and 27 of the official action, the examiner rejected claims 9, 12, 13, 15, 16, 18, 21, and 22 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. Specifically, the examiner asserted that the phrase "the genes as SEQ ID NO: 1 and encoding SEQ ID NO:2" is confusing, particularly in light of the definition of overexpression in the specification on page 10. The examiner also asserted that it was unclear what was being enriched in step (b) of claim 9 because cells will grow without assistance upon culturing.

Amended claim 9 is now directed to a process for the production of an L-amino acid comprising culturing a coryneform bacterium under conditions suitable for overexpression of the *sig*E gene having the nucleic acid sequence as set forth in SEQ ID NO: 1 and isolating the L-amino acid wherein overexpression occurs by increasing the copy number of said gene or operatively linking said gene to a promoter. The phrase "and encoding a polypeptide having an amino acid sequence as set forth in SEQ ID NO: 2" has been removed thereby obviating the rejection. In addition, as suggested by the examiner and for which the applicants are grateful, step (b) of claim 9 has been removed thereby obviating this part of the rejection. Claims 12, 13, 15, 16, 18, 21, and 22 are directly or indirectly dependent upon claim 9 and thereby the rejection of these claims has been overcome as well. In view of the foregoing amendment, the applicants respectfully request the rejection of claims 9, 12, 13, 15, 16, 18, 21, and 22 under 35 U.S.C. §112, second paragraph, has been overcome and should be withdrawn.

Rejection Under 35 U.S.C. §112, First Paragraph

Written Description

In paragraph 29 of the official action, the examiner rejected claims 15 and 16 under 35 U.S.C. §112, first paragraph, for allegedly lacking proper written descriptive support. Specifically, the examiner alleged a protein for lysine export, Zwa1 protein, and Zwa2 protein do not indicate a structure/function relationship as found with the other specific enzymes noted in the claims. Specifically, the examiner asserted there was no description of how to maintain Zwa1-like protein structures and/or function, and thus one of skill would be unable to predict the structure of other members of the genus of genes claimed.

As discussed above, claims 15 and 16 have been amended whereby reference to the Zwa1 and Zwa2 proteins has been removed. With reference to the gene encoding a protein

that exports lysine in part (i) of claim 15, the specification clearly indicates this gene is the *lys*E gene on page 13, lines 10 and 11. The *lys*E gene was known in the art before filing this applications as a transporter protein in *C. glutamicum* that specifically exported L-lysine (*See* Attachment B, *Mol. Microbiol.*, abstract). Amended claim 15 is directed to overexpressing particular *C. glutamicum* genes (*i.e.*, the *lys*E gene) in combination with the *sig*E gene. Accordingly, the specification provides a specific description of the structure and function of the *C. glutamicum* gene which encodes a protein for lysine export. In view of the foregoing amendment and remarks, the applicants submit the rejection of claims 15 and 16 under 35 U.S.C. §112, first paragraph, for lacking proper written description, has been overcome and should be withdrawn.

Enablement

In paragraph 30 of the official action, the examiner rejected claims 9, 12, 15, 16, 18, and 21-28 under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement. Specifically, the examiner asserted that while the specification was enabling for overexpressing SEQ ID NO: 1 by transforming a host cell with a vector comprising SEQ ID NO:1 and a promoter wherein the promoter is responsible for overexpression, the specification does not provide enablement for overexpressing SEQ ID NO: 1 by means otherwise mentioned in the specification.

In paragraph 31 of the official action, the examiner further rejected claims 15 and 16 of the official action under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement. Specifically, the examiner alleged that while the specification was enabling for methods using known *C. glutamicum zwa*1, *zwa*2, and *lys*C genes as described in the specification, it does not provide enablement for methods using other *zwa*1, *zwa*2, and *lys*C genes.

With regard to the enablement rejection of the term "overexpression" in claims 9, 12, 15, 16, 18, and 21-28, the applicants have amended claim 9 as discussed above. Specifically, claim 9 is now directed to the modes of overexpression the *C. glutamicum sig*E gene set forth in SEQ ID NO: 1 including increasing the copy number of the *sig*E gene or operatively linking the *sig*E gene to a promoter, which the examiner acknowledges as enabled (See Office Action, paragraph 30).

As discussed above, the applicants submit the terms Zwa1 and Zwa2 have been removed from claim 15 or 16 without prejudice and solely to expedite prosecution. With regard to the rejection of the term *lys*C, it is unclear to the applicants why *lys*C is part of this

rejection. The specification clearly teaches *C. glutamicum's lys*C gene encodes aspartate kinase and can be used in the claimed methods (see specification on page 13, lines 8 and 9).

The applicants are wondering whether the examiner meant to discuss possible enablement issues with regard the *lys*E gene rather than the *lys*C gene. Assuming this presumption, the applicants submit that part (j) of claim 15 is fully enabled as one of skill in the art knew (at the time of filing) the *lys*E gene from *Corynebacterium glutamicum* encodes a translocator that specifically exports L-lysine from the cell. In addition, as the examiner has acknowledged in paragraph 31 of the official action, the claimed methods for using the known *C. glutamicum lys*E gene is enabled by the specification. In view of the foregoing amendment and remarks, the applicants submit the rejection of claims 15 and 16 under 35 U.S.C. §112, first paragraph, for lack of enablement, has been overcome and should be withdrawn.

CONCLUSION

In view of the foregoing, the claims are now believed to be in form for allowance, and such action is hereby solicited. If any point remains at issue which the examiner feels may be best resolved through a personal or telephone interview, the examiner is strongly urged to contact the undersigned at the number listed below.

Respectfully submitted,
PILLSBURY WINTHROP LLP

By: _____
THOMAS A. CAWLEY, JR., Ph.D.
Reg. No. 40944
Tel. No. (703) 905-2144
Fax No. (703) 905-2500

TAC/PAJ/wks
P.O. Box 10500
McLean, VA 22102
(703) 905-2000

ABSTRACT

The present invention relates to an isolated polynucleotide from *Corynebacterium glutamicum* comprising a polynucleotide sequence chosen from the group consisting of (a) a polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID NO: 2; (b) a polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID NO: 2; (c) a polynucleotide which is complementary to the polynucleotides of (a) or (b); and (d) a polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of (a), (b), or (c), and a process for the fermentative preparation of L-amino acids using coryneform bacteria in which at least the sigE gene is present in enhanced form, and the use of polynucleotides which comprise the sequence according to the invention as hybridization probes.

ATTACHMENT A

LOCUS P38133 177 aa linear BCT 15-JUN-2004
DEFINITION RNA polymerase sigma-E factor.
ACCESSION P38133
VERSION P38133 GI:585924
DBSOURCE swissprot: locus RPOE_STRCO, accession P38133;
class: standard.
created: Oct 1, 1994.
sequence updated: Oct 1, 1994.
annotation updated: Jun 15, 2004.
xrefs: gi: 532250, gi: 532251, gi: 3980219, gi: 3980221, gi: 24413781, gi: 4585588, gi: 7481805
xrefs (non-sequence databases): InterProIPR009043, InterProIPR000838, InterProIPR007627, InterProIPR007630, PfamPF04542, PfamPF04545, PROSITEPS01063
KEYWORDS Transcription regulation; Sigma factor; DNA-directed RNA polymerase; DNA-binding; Complete proteome.
SOURCE Streptomyces coelicolor
ORGANISM Streptomyces coelicolor
Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Streptomycineae; Streptomycetaceae; Streptomyces.
REFERENCE 1 (residues 1 to 177)
AUTHORS Lonetto,M.A., Brown,K.L., Rudd,K.E. and Buttner,M.J.
TITLE Analysis of the Streptomyces coelicolor sigE gene reveals the existence of a subfamily of eubacterial RNA polymerase sigma factors involved in the regulation of extracytoplasmic functions
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 91 (16), 7573-7577 (1994)
MEDLINE 94329558
REMARK SEQUENCE FROM N.A., AND SEQUENCE OF 1-27.
STRAIN=A3(2)
REFERENCE 2 (residues 1 to 177)
AUTHORS Paget,M.S., Leibovitz,E. and Buttner,M.J.
TITLE A putative two-component signal transduction system regulates sigmaE, a sigma factor required for normal cell wall integrity in Streptomyces coelicolor A3(2)
JOURNAL Mol. Microbiol. 33 (1), 97-107 (1999)
MEDLINE 99340542
REMARK SEQUENCE FROM N.A.
STRAIN=A3(2)
REFERENCE 3 (residues 1 to 177)
AUTHORS Bentley,S.D., Chater,K.F., Cerdeno-Tarraga,A.M., Challis,G.L., Thomson,N.R., James,K.D., Harris,D.E., Quail,M.A., Kieser,H., Harper,D., Bateman,A., Brown,S., Chandra,G., Chen,C.W., Collins,M., Cronin,A., Fraser,A., Goble,A., Hidalgo,J., Hornsby,T., Howarth,S., Huang,C.H., Kieser,T., Larke,L., Murphy,L., Oliver,K., O'Neil,S., Rabbinowitsch,E., Rajandream,M.A., Rutherford,K., Rutter,S., Seeger,K., Saunders,D., Sharp,S., Squares,R., Squares,S., Taylor,K., Warren,T., Wietzorrek,A., Woodward,J., Barrell,B.G., Parkhill,J. and Hopwood,D.A.
TITLE Complete genome sequence of the model actinomycete Streptomyces

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  JOURNAL   Nature 417 (6885), 141-147 (2002)
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            collaboration between the Swiss Institute of Bioinformatics and
            the EMBL outstation - the European Bioinformatics Institute.
            The original entry is available from http://www.expasy.ch/sprot
            and http://www.ebi.ac.uk/sprot
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            then released. This sigma factor is involved in the transcription
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Entrez Protein

Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Boo

Search Protein for

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REFERENCE   1  (residues 1 to 257)
  AUTHORS   Cole,S.T., Brosch,R., Parkhill,J., Garnier,T., Churcher,C.,
            Harris,D., Gordon,S.V., Eiglmeier,K., Gas,S., Barry III,C.E.,
            Tekaia,F., Badcock,K., Basham,D., Brown,D., Chillingworth,T.,
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            Rajandream,M.A., Rogers,J., Rutter,S., Seeger,K., Skelton,S.,
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            Barrell,B.G.
  TITLE     Deciphering the biology of Mycobacterium tuberculosis from the
            complete genome sequence
  JOURNAL   Nature 393 (6685), 537-544 (1998)
  MEDLINE   98295987
   PUBMED   9634230
REFERENCE   2  (residues 1 to 257)
  AUTHORS   NCBI Microbial Genomes Annotation Project.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-JUN-2001) National Center for Biotechnology
            Information, NIH, Bethesda, MD 20894, USA
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
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                     extracytoplasmic function sigma factor involved in
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survival following stress; J. Bacteriol.
179:2922-2929(1997). Similar tomany eg. RPOE_HAEIN P44790
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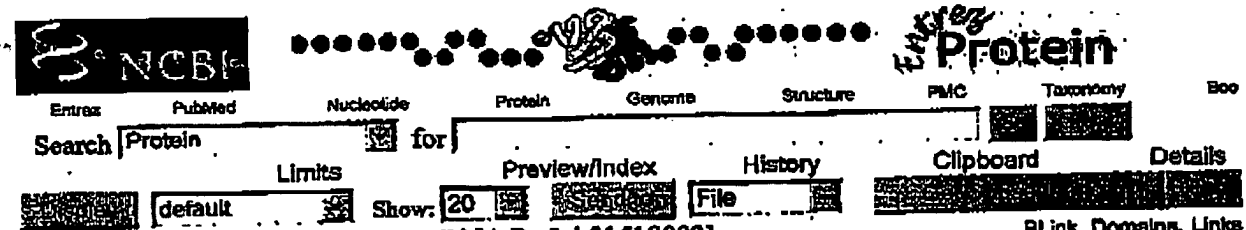

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VERSION CAD94114.1 GI:31618003
DBSOURCE embl accession BX248338.1
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ORGANISM Mycobacterium bovis AF2122/97
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REFERENCE 1
AUTHORS Garnier,T., Eiglmeier,K., Camus,J.-C., Medina,N., Mansoor,H., Pryor,M., Duthoy,S., Grondin,S., Lacroix,C., Monsempe,C., Simon,S., Harris,B., Atkin,R., Doggett,J., Mayes,R., Keating,L., Wheeler,P.R., Parkhill,J., Barrell,B.G., Cole,S.T., Gordon,S.V. and Hewinson,G.
TITLE The complete genome sequence of Mycobacterium bovis
JOURNAL Unpublished
REFERENCE 2 (residues 1 to 257)
AUTHORS Garnier,T.
TITLE Direct Submission
JOURNAL Submitted (24-MAR-2003) Garnier T., Unite de Genetique Moleculaire Bacterienne Institut Pasteur 28,rue du Dr Roux 75724 PARIS cedex 15, France. e-mail:tgarnier@pasteur.fr Submitted on behalf of the Mycobacterium bovis sequencing teams, TB Research Group, Veterinary Laboratories Agency Weybridge, Woodham Lane, New Haw,Addlestone, Surrey KT15 3NB, UK. Sanger Centre, Wellcome Trust Genome Campus, Hinxton,Cambridge CB10 1SA, UK. PT4 Annotation, Genopole, Institut Pasteur, 28 Rue du Docteur Roux, 75724 Paris Cedex 15, France. Unite de Genetique Moleculaire Bacterienne, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

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RNA polymerase sigma-e factor from Haemophilus influenzae (189 aa), FASTA scores: opt: 247, E(): 3.4e-06, (28.5% identity in 186 aa overlap); etc. Also similar to MTCY07D11.03 rpoE from Mycobacterium tuberculosis (35.2% identity in 159 aa overlap). BELONGS TO THE SIGMA-70 FACTOR FAMILY, ECF SUBFAMILY. Note that in Mycobacterium bovis BCG, the sigE gene is transcribed from two promoters, P1 and P2, and that these promoters were expressed at temperatures from 30-50 C."
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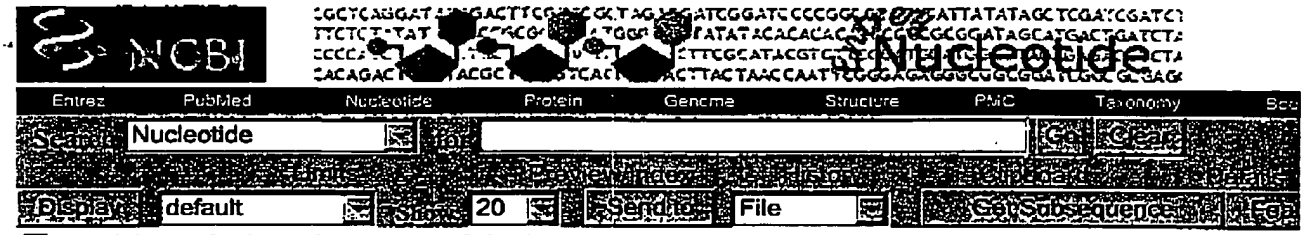

☐ 1: X96471. C.glutamicum lysE...[gi:1729753] Links

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ACCESSION   X96471
VERSION     X96471.1  GI:1729753
KEYWORDS    lysE gene; lysG gene; Lysine export regulator protein; Lysine
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SOURCE      Corynebacterium glutamicum
  ORGANISM  Corynebacterium glutamicum
            Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
            Corynebacterineae; Corynebacteriaceae; Corynebacterium.
REFERENCE   1
  AUTHORS   Vrljic,M., Sahm,H. and Eggeling,L.
  TITLE     A new type of transporter with a new type of cellular function:
            L-lysine export from Corynebacterium glutamicum
  JOURNAL   Mol. Microbiol. 22 (5), 815-826 (1996)
  MEDLINE   97126810
   PUBMED   8971704
REFERENCE   2  (bases 1 to 2374)
  AUTHORS   Vrljic,M.M.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-MAR-1996) M.M. Vrljic, Institut fuer Biotechnologie
            1, Forschungszentrum Juelich, Postfach 1913, D-52425 Juelich, FRG
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ATTACHMENT B

1: Mol Microbiol. 1996 Dec;22(5):815-26. Related Articles, Links

A new type of transporter with a new type of cellular function: L-lysine export from Corynebacterium glutamicum.

Vrljic M, Sahm H, Eggeling L.

Institut fur Biotechnologie, Forschungszentrum Julich GmbH, Germany.

We discovered that after deregulation of the L-lysine biosynthesis in Corynebacterium glutamicum, L-lysine accumulated in the cytosol and the efflux properties of this amino acid in mutants used for L-lysine production were altered. In this study we describe the cloning and molecular identification of lysE, which encodes the translocator specifically exporting L-lysine from the cell. The lysE gene product does not display homology to any known transporter. It is only 236 amino acids in size, with the potential to span the membrane six times. The LysE protein was oversynthesized to confirm its deduced M(r) of 25425 Da. A probable regulatory gene, lysG, is localized immediately adjacent to lysE and displays all the typical structural features of an autoregulatory transcriptional regulator of the LysR-type family. L-Lysine export is correlated with lysE expression. A null mutant is unable to excrete L-lysine, whereas with overexpressed lysE, L-lysine is exported at a rate of 3.76 nmol min-1 mg-1 dry weight, which is five times the rate that was obtained with the wild type. A deletion mutant was constructed to search for a natural function of this unique carrier. Surprisingly, growth of this mutant is abolished on a salt medium in the presence of the dipeptide Lys-Ala. The quantification of the intracellular L-lysine concentrations revealed that, in response to peptide addition, there was an accumulation of the exceptionally high concentration of more than 1100 mM L-lysine. These results distinguish LysE as an exporter, which: (i) structurally represents a new type of translocator; (ii) demonstrates that exporters are also present for primary metabolites such as amino acids; and (iii) serves in one physiological function to link import with export activity.

MeSH Terms:

- Amino Acid Sequence
- Bacterial Proteins/genetics
- Bacterial Proteins/metabolism*
- Base Sequence
- Carrier Proteins/genetics
- Carrier Proteins/metabolism*
- Cloning, Molecular
- Corynebacterium/genetics
- Corynebacterium/growth & development
- Corynebacterium/metabolism*
- DNA, Bacterial
- Genetic Complementation Test
- Lysine/metabolism*
- Molecular Sequence Data
- Sequence Analysis, DNA
- Support, Non-U.S. Gov't

Substances:

- Bacterial Proteins
- Carrier Proteins
- DNA, Bacterial
- LysB protein, Corynebacterium glutamicum
- Lysine

Secondary Source ID:

- GENBANK/X96471

PMID: 8971704 [PubMed - indexed for MEDLINE]

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